GLOMALIN PURIFICATION
PRECIPITATION AND DIALYSIS*

(Wright *et al.*,1998)

Introduction

Precipitation and dialysis are used to purify glomalin prior lyophilyzation (freeze-drying) and storage. This also helps to purify and concentrate the protein for subsequent analysis. Protein concentration should be at least 5 mg or the freeze-dried amount will be rather small and difficult to measure accurately. Several different chemicals may be used to precipitate and reconstitute glomalin which may be dialyzed against a dilute buffer solution (such as borate) or water. Like other proteins, glomalin will precipitate in a high ionic (salt) solution such as NH_4SO_4. Glomalin is insoluble in acid solution and will precipitate in HCl at pH 2.0 to 2.5. In addition, glomalin may be reconstituted in many alkaline solutions such as NaOH for dialysis.

Materials

Precipitation solutions*: (1) 20% TCA (Trichloroacetic acid), (2) 1 N HCl, or (3) 4 M NH_4SO_4
Reconstitution solutions*: (1) 100mM sodium borate, pH 9.0, or (2) 0.1 M NaOH
Dialysis solutions*: (1) 10 mM sodium borate, pH 8.0, or (2) deionized (Milli-Q) water (dH_2O)

Dialysis tubing (≤12,000 Daltons) and clamps
Dialysis jar, large beaker or bucket (5L container), thin foam circle that fits in top
Centrifuge and centrifuge tubes
Ice and ice bucket
Pasteur pipettes and bulbs
Vacuum, freeze drier

*Note: Several different chemicals may be used in this procedure depending on experimental needs. There is a possibility that some of the TCA or NH_4SO_4 will adhere to glomalin during these procedures. If you are measuring carbon and nitrogen concentrations in glomalin, this may produce inaccurate results. In this case, it is recommended that you use HCl and NaOH to eliminate this problem.

Methods

Precipitation:

1) In centrifuge tubes, (1) mix samples 1:1 with ice cold 20% TCA or with 4 M NH_4SO_4 or (2) titrate samples to pH 2.0 to 2.5 with 1 N HCl.
2) Incubate in ice for 1 hr.
3) Balance tubes and centrifuge at 10,000 for 10 min.
4) Remove supernatant.

Reconstitution:

1) Add 100mM borate* at pH 9.0 or 0.1 M NaOH in 1-ml increments to bring precipitate into solution.
2) Use Pasteur pipette to mix solution and try to minimize volume.
3) Make sure that all of the precipitate has been reconstituted.

*Note: Make borate solution 1-2 hrs prior, because it is difficult to dissolve.

Dialysis:

1) Dialysis solution: Fill dialysis jar, beaker or bucket with 10mM borate at pH 8.0 (500 ml 100 mM borate + 4500 ml water) or dH_2O.
2) Prepare dialysis tubing: Cut a strip of dialysis tubing (1.5X the amount needed to hold the reconstituted sample). Soak the piece of tubing in a large volume of dH_2O to remove glycerine, clamp bottom, and rub other end between fingers to open.
3) Transfer sample into bags using Pasteur pipette and place bags in dialysis solution, cover with a thin piece of foam to keep bags immersed in solution, and incubate for at least 8 hr while stirring constantly.
4) Change dialysis solution at least five times (more if a large volume is being dialyzed) at 6 to 12 h intervals.
5) Remove bags from solution and transfer contents into centrifuge tubes.
6) Centrifuge at 10,000 rpm for 10 min.
7) Collect supernatant and freeze at $-20^{o}C$ for 1+ hr (until completely frozen). Place in vacuum freeze drier until dry.